Iranian Journal of Veterinary Science and Technology
Vol. 5, No. 2, 2013, 62-69

IJVST

Determination of *Feline calicivirus* in cats in Ahvaz district, Southwest of Iran by RT-PCR (a preliminary study)

Reza Avizeh[1], Masoud Reza Seyfi Abad Shapouri[2],
Bahman Mosallanejad[1] , Fatameh Faridan Esfahani[3]

[1] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
[2] *Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
[3] *Graduated from Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*

Received: November 29, 2012 Accepted: September 24, 2013

Abstract

Feline calicivirus (FCV) is a highly infectious respiratory pathogen of domestic cats with a widespread distribution. In order to assess how FCV circulates in feral and household cats, we have carried out the first study on the FCV detection in Ahvaz district from December 2008 to November 2009. Oropharyngeal, nasal and ocular swabs of one hundred cats (70 feral and 30 household) were evaluated by reverse transcription polymerase chain reaction (RT-PCR) procedure for the detection of FCV. The influence of sex, age, social status and clinical signs on the probability of infection was analyzed using statistical Fisher's exact test. Overall, feline calicivirus was detected in 4/100 (4%) of sampled cats; 13.3% of the household cats were FCV positive compared to 0% of feral cats. According to several factors including younger cats (under 6 months of age), multiple cat household and clinical findings, differences were significant (p<0.05) in statistical analysis. There was no significant difference between the sex distributions of the cats (p>0.05). To the best of our knowledge, this is the first report indicating the presence of FCV in cats in Iran. Due to low prevalence of FCV infection and the fact that feral cats live solitarily, it was concluded that this viral infection don't spread readily within feral populations. However special measures are recommended to avoid infection of susceptible and unvaccinated cats.

Keywords: *Feline calicivirus*, Feline viral respiratory infections, Iran, RT-PCR

Corresponding author: Reza Avizeh
Email: avizeh@scu.ac.ir
Tel: +98 611 3738163
Fax: +98 611 3360807

Introduction

Feline calicivirus (FCV) is a highly infectious pathogen of wild and domestic cats with a widespread distribution in the feline population. The virus typically causes moderate, self-limiting acute oral and upper respiratory tract disease. Feline calicivirus vaccines have been used widely in the field over the past 20 years. Despite these efforts, feline calicivirus associated diseases still present as a major problem in the feline population especially for cats living in groups (Binns *et al.*, 2000 and Dawson *et al.*, 2001). The viruses are mainly shed in ocular, nasal and oral secretions, and spread largely by direct contact (Coyne *et al.*, 2006). Diagnosis may be attempted based on clinical signs alone. Confirmatory diagnosis of FCV can be made by virus isolation in feline cell cultures, immunofluorescence or enzyme-linked immunosorbent assay (ELISA) techniques, or RT-PCR on oropharyngeal or conjunctival swabs. Serology is generally not helpful in the diagnosis of FCV infection because of widespread antibodies resulting from vaccination (Marsilio *et al.*, 2005 and Greene, 2006). The prevalence of FCV has been frequently reported in cats throughout the world (Sykes *et al.*, 2001; Cai *et al.*, 2002; Bannasch and Foley, 2005 and Zicola *et al.*, 2009). The prevalence of healthy, FCV-positive animals has been described in the range between 15% and 25% (Harbour *et al.*, 1991 and Coutts *et al.*, 1994). The purpose of this study was to identify the prevalence of FCV in feral and household cats in Iran by performing reverse transcription-polymerase chain reaction (RT-PCR). To the best of our knowledge, this is the first report of calicivirus detection in cat population in Iran.

Materials and methods

The present study was performed to determine the prevalence of FCV infection in cats of Ahvaz district, Southwest of Iran from December 2008 to November 2009. Specimens were obtained from ocular conjunctiva, nose and oropharynx of 100 cats (70 feral and 30 household). All of the studied cats were domestic short hair (DSH). Classification was made by age, sex and clinical signs. The studied cats were divided into two groups based on age (<6 months, and >6 months). A thorough clinical examination was conducted for all of the studied cats.

For each cat, specimens were collected from conjunctival sacs of the eyes, nostrils and oropharynx respectively, using three sterile cotton tipped swabs. The samples were sent to the Laboratory of Virology, School of Veterinary Medicine of Ahvaz for RT-PCR testing. The swabs were preserved in 1.5 ml DMEM culture media (Bahar Afshan Co., Tehran, Iran) containing antibiotic and they were immediately sent to the laboratory within 2 hours. Before the subsequent nucleic acid extraction, the specimens, which were separately obtained from the three sites, were thoroughly mixed. RNA extraction from the samples was performed by using Tripure, a commercial RNA extraction solution (Roche, Germany) as described previously (Seyfi Abad Shapouri *et al.*, 2004). FCV strain F9, cultured in the feline embryo fibroblast cell line (FEA) was used as positive control. Sterile distilled water without template was also used as negative control. Both controls were subjected to nucleic acid extraction and RT-PCR. One pairs of oligonucleotide primers were used for the amplifying reaction. We used the previous primer sequences designed by Sykes *et al.* (1998), CalcapF (5'-TTCGGCCTTTTGTGTTCC-3') and Calcap R (5'-TTGAGAATTGAACACATCAATAGATC-3'), to amplify a 673-bp conserved region in the capsid protein gene of FCV. RT and PCR reactions were performed according to previous study (Sykes *et al.*, 2001). 10 µl of each reaction product was electrophoresed through a 1.5% agarose gel and the DNA bands were stained with ethidium bromide.

Results

The detection of FCV infection was 4% (4 out of 100) in the studied cats indicating that this

virus is present in cat population of Ahvaz district. The infection had more detection in young cats less than 6 months (4 out of 34; 11.8%) in comparison with cats above 6 months (0 out of 66; 0%), and the difference was significant (p<0.05) (Table 1). There was no significant difference between different sexes, although the detection rate was higher in male cats (4.3%; 2 out of 47) than females (3.8%; 2 out of 53) (p>0.05) (Table 2). The infection had more prevalence in household cats with ocular and upper respiratory tract disease (28.6%; 4 of 14) compared with cats without clinical signs (0%; 0 of 16) and the difference was significant (p<0.05). All of the positive cats were from domestic short-haired, weighing 1250-1950 g and had a history of pyrexia, anorexia, depression, oral ulceration, conjunctivitis, sneezing, and ocular and nasal discharges. In addition, increased respiratory (40-44/min), pulse rates (245-278/min), and mild pale mucosa were observed at physical examination. Rectal temperature was up to 40.1°C. Ulcerations were only on the tongue and they showed hypersalivation with moisture on the fur around the mouth, but no drooling of saliva. There was no skin ulcerations, dyspnea, pneumonia, lameness, facial and paw edema, icterus, nasal hemorrhage and bloody feces, which previously reported as virulent systemic disease associated with FCV. They were from outdoor private household cats maintained in the same building, but their parents were among the feral cats. All of the positive cats had no history of routine vaccination. Initial diagnosis made based on clinical signs alone, especially oral ulceration was suggestive FCV infection. Detection of FCV RNA by RT-PCR confirmed FCV presence in pooled oral, nasal and ocular secretions (Fig. 1).

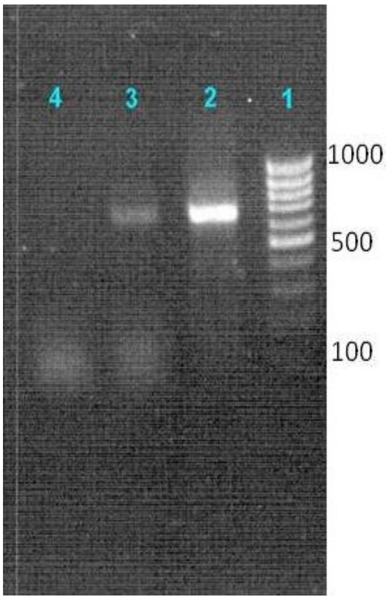


Figure 1. Agar gel electrophoresis analysis of *Feline calicivirus* RT-PCR products. Lane 1: 100 bp DNA ladder(100, 500 and 1000 base pairs bands are indicated at the right); Lane 2: Positive control (FCV strain F9); Lane 3: One of the FCVpositive samples; Lane 4: Negative control (no template control)

Table 1. Determination of F*eline calicivirus* infection in cats of different age and sex in Ahvaz district, Southwest of Iran by RT-PCR, 2008-2009.

Age	< 6 months		> 6 months	
Sex	Negative	Positive	Negative	Positive
Male	13	2	32	0
Female	17	2	34	0
Total = 100	30	4	66	0

Table 2. Determination of *Feline calicivirus* infection in cats of different age and region in Ahvaz district, Southwest of Iran by RT-PCR, 2008-2009.

Age	< 6 months		> 6 months	
Region	**Negative**	**Positive**	**Negative**	**Positive**
South	4	1	12	0
West	8	0	17	0
East	7	2	14	0
Central	5	1	13	0
Total	6	0	10	0

Discussion

The present study, as the first report on detection of FCV in cats in Ahvaz district, Southwest of Iran, revealed that FCV was detected in 4% of the studied cats. Likewise FCV was rarely detected in a study that focused on clinically normal cats (2.6% in Sweden) using cell culture (Holst *et al.*, 2005). Other previous studies even showed that FCV was not detected from clinically abnormal cats in a Korean animal shelter by RT-PCR (Kang and Park, 2008). Results of low prevalence studies may be due to several reasons. First, it is likely that all of the studied cats were not truly infected with FCV. The second possible reason is that the chronic infected cats were not shedding virus (Kang and Park, 2008). Due to low prevalence of FCV detection and the fact that feral cats live solitarily, it was concluded that, this viral infection doesn't spread readily within feral populations. These cats may play an important role in transmission of infection to other cats especially because some individual carriers may shed virus for life (Radford *et al.*, 2007). However special measures are recommended to avoid infection of susceptible and unvaccinated cats.

In the present study, the positive cats had pyrexia, loss of appetite, tongue ulcerations, conjunctivitis, sneezing, and nasal and ocular discharges. A range of clinical signs may be seen in the affected cats, due to the large number of different strains of FCV. The most characteristic lesion is oral ulceration. Ocular and nasal discharges also frequently occur. Calicivirus strains can also cause an acute febrile lameness syndrome which has been recreated experimentally (Pedersen *et al.*, 1983 and Radford *et al.*, 2007). In some occasions, kittens with low levels of maternally derived antibody (MDA) may become subclinically infected and become latent carriers without showing clinical signs (Povey and Ingersoll, 1975).

In our study, all of the positive cats lived together in a house. They were outdoor private household kittens that originate from feral cats. Feline calicivirus infection is widespread in the general cat population (Binns *et al.*, 2000 and Mochizuki *et al.*, 2000). The prevalence is generally broadly proportional to the number of cats in the household, with the highest prevalence usually seen where large groups of cats are housed together. As a result, privately owned pet cats kept in small numbers generally have relatively low prevalence approximately 10% (Wardley *et al.*, 1974). In contrast, random cats living in colonies or shelters usually have a higher chance of being infected from 25% to 40% (Radford *et al.*, 2001 and Bannasch and Foley, 2005).

In accordance with other previous studies, cats less than 6 months old (4%) were at significantly greater risk than older cats (0%) (Binns *et al.*, 2000 and Yagami *et al.*, 1985). However, the sources of infection in these kittens were not clear. They may became infected from their parents or another feral cats, which coming to their home for food consuming several times a day. Isolation rates of FCV have been shown to be higher in young (less than 1 year old) than in older animals (Harbour *et al.*, 1991; Wardley *et al.*, 1974 and Coutts *et al.*, 1994). MDAs against FCV may persist in kittens for 10 to 14 weeks. It is generally accepted that FCV tends to occur in young kittens as they lose their

maternally-derived antibody (Povey and Ingersoll, 1975).

Based on results of this study between the sexes, the difference was not statistically significant. No specific patterns were found for FCV as for gender distribution of those cats in different areas of the United Kingdom (Cave *et al.*, 2002; Binns *et al.*, 2000, Harbour *et al.*, 1991 and Knowles *et al.*, 1989).

In the present survey, all studied cats, including four FCV positive cats, belonged to the domestic shorthair breeds. Coutts *et al.* (1994) found a significant difference between breeds in their study, with the highest prevalence of FCV-positive cats in the longhair breeds. Higher prevalence within certain breeds may point to an increased susceptibility for infection, differences in environment and management, or simply the fact that once introduced within a breed, virus spread occurs more efficiently, due to closer contact between cattery cats of the same breed (Radford *et al.*, 2007).

Vaccination against FCV usually reduces clinical signs (Chomel *et al.*, 1995) but does not eliminate clinical illness (Harbour *et al.*, 1991). Furthermore, vaccination does not protect against the chronic carrier state (Chomel *et al.*, 1995). In the present study, all the FCV-positive cats showed signs of clinical illness, and were not vaccinated. It was concluded that due to the presence of FCV in the environment, special measures are recommended to avoid infection of susceptible cats. In conclusion more investigations are needed to be conducted in this regard in order to clarify the epidemiological picture of feline respiratory pathogens in Iran. Booster vaccination of queens is recommended to ensure high levels of maternal antibodies in the colostrum (Lappin *et al.*, 2006). Queens with kittens should also be kept separately, to reduce the risk of other cats in the cattery infecting the kittens. In breeding catteries where respiratory tract disease is a problem, kittens can be vaccinated from 6 weeks of age (Dawson *et al.*, 2001).

Acknowledgements

This study was supported financially by the Research Council of veterinary college-Shahid Chamran University of Ahvaz, for which the authors are most grateful. We thank Dr. Karen Coyne from the University of Liverpool, UK, for providing us the viral strains and the cell line.

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Iranian Journal of Veterinary Science and Technology
Vol. 2, No. 2, 2010, 62-69

IJVST

تعیین کلیسی ویروس گربه در گربه‌های منطقه اهواز، جنوب غرب ایران به روش واکنش زنجیره‌ای پلیمراز معکوس (یک مطالعه مقدماتی)

رضا آویزه[۱]، مسعود رضا صیفی آباد شاپوری[۲]، بهمن مصلی نژاد[۱]، فاطمه فریدن اصفهانی[۳]

[۱] گروه علوم درمانگاهی دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز ،ایران
[۲] گروه پاتوبیولوژی دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز ،ایران
[۳] دانش آموخته دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز ،ایران

دریافت مقاله : ۱۳۹۱/۰۹/۱۹ پذیرش نهایی:۱۳۹۲/۰۷/۰۲

چکیده

کلیسی ویروس گربه یک پاتوژن فوق‌العاده عفونی دستگاه تنفس گربه‌ها با انتشار جهانی می‌باشد. مطالعه حاضر جهت تعیین چگونگی انتشار عفونت ناشی از کلیسی ویروس در گربه‌های منطقه اهواز و فاصله زمانی آذر ۱۳۸۷ لغایت آبان ۱۳۸۸ انجام شده بود. به منظور تعیین عفونت کلیسی ویروس سواب‌های گرفته شده از ناحیه دهان و حلق، مخاط بینی و ملتحمه چشم ۱۰۰ قلاده گربه (۷۰ قلاده ولگرد و ۳۰ قلاده خانگی) به روش واکنش زنجیره پلیمراز معکوس مورد ارزیابی قرار گرفتند. تأثیر جنس، سن، وضعیت نگهداری و وجود علائم بالینی بر احتمال عفونت، با استفاده از روش آماری دقیق فیشر تجزیه و تحلیل شدند. در کل میزان عفونت ناشی از کلیسی ویروس در گربه‌های مورد مطالعه ۴ درصد تعیین گردید، در حالی‌که ۱۳/۳ درصد گربه‌های خانگی در مقایسه با صفر درصد گربه‌های ولگرد از این نظر مثبت بودند. به دنبال ارزیابی آماری، چندین فاکتور از جمله میزان عفونت در گربه‌های جوان کمتر از ۶ ماه، زندگی دسته‌جمعی و وجود نشانی‌های درمانگاهی اهمیت معنی‌داری را دارا بودند ($P<0.05$). از طرفی هیچ اختلاف معنی‌داری بین میزان شیوع بیماری و توزیع گربه‌ها بر اساس جنس نبود ($P>0.05$). بر طبق اطلاعات، این اولین گزارش مبنی بر حضور عفونت ناشی از کلیسی ویروس در گربه‌های ایران است. به دلیل شیوع پایین کلیسی ویروس گربه و زندگی انفرادی گربه‌های ولگرد، می‌توان نتیجه گرفت که عفونت کلیسی ویروس به آسانی در جمعیت گربه‌های ولگرد گسترش پیدا نمی‌کند. با این وجود، اقدامات مدیریتی اختصاصی برای اجتناب از آلودگی گربه‌های مستعد و واکسینه نشده پیشنهاد می‌شود.

واژگان کلیدی: کلیسی ویروس گربه، عفونت‌های تنفسی ویروسی گربه، ایران، واکنش زنجیره‌ای پلیمراز معکوس